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Glucose-6-phosphate Dehydrogenase Deficiency and Severity of Neonatal Jaundice: a Prospective Study from Port-Harcourt

IO George*, NA Akani**, RS Oruamabo***

Abstract

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Background: Port-Harcourt, capital of Rivers state of Nigeria, is the centre of an oil exploration industry. Babies born in the environment could stand an increased risk of exposure to iterogenic agents. Infants who are glucose-6-phosphate dehydrogenase (G6PD) deficient may develop jaundice when exposed to these noxious substances. The aim of this studywas to determine the severity of jaundice among G6PD deficient neonates at the University of Port-Harcourt Teaching Hospital, Port-Harcourt (UPTH).

Methods: A prospective study of 400 jaundiced neonates admitted into the Special Care Baby Unit (SCBU) of the UPTH from June 1 to December 31, 2006, was carried out. Physical examination of all the babies admitted, with special reference to jaundice, was done. A structured questionnaire was used to collect data on mother and neonate; such data included sociodemographics, exposure to iterogenic substances, history of peripartum pyrexia, prolonged rupture of foetal membranes, age at onset of jaundice, history of seizure, mode of treatment of jaundice and outcome. Two mls of venous blood withdrawn from each neonate was put in an EDTA bottle and immediately sent to the laboratory for quantitative G6PD enzyme assay based on the method of Kornberg. Serum bilirubin level was estimated using the Van den Bergh diazo reaction. Data were arranged in frequency tables and discrete variables were compared using Chi-square statistic, while continuous variables were compared using the Student t test. In all instances, a p value of less than 0.05 was regarded as statistically significant.

Results: Out of the 400 jaundiced neonates, 210 (52.5 percent) were G6PD deficient, while 190 (47.5 percent) had normal G6PD activity. There were 145 (69.0 percent) G6PD deficient males and 65 (31.0 percent) females. One hundred and thirty five (64.3 percent) of the G6PD deficient neonates were severely jaundiced (106 males and 29 females). A greater proportion of those with severe jaundice were males (X^2 = 14.65; df; p = 0.0013). Eighty four (42.0 percent) of the G6PD deficient neonates were exposed to iterogenic substances. Sixty eight (81.0 percent) of those so exposed were severely jaundiced, while the jaundice was not severe in 16 (19.0 percent). The association between exposure to iterogenic substances and severity of jaundice was statistically significant (X^2 = 15.75; df = 1; p=0.00072). Clinical features of kernicterus were found in seven (3.3 percent) of the deficient neonates while 15 (7.1 percent) died.

Conclusion: G6PD deficiency is commonly associated with severe neonatal jaundice, the severity of which is influenced by gender and exposure to iterogenic substances. It is recommended that any neonate presenting with jaundice should be screened for G6PD status not only to define the aetiology of hyperbilirubinaemia but also to prevent future haemolytic episodes.

Key words: Glucose-6-phosphate dehydrogenase deficiency, severe jaundice, neonate

University of Port-Harcourt Teaching Hospital, Port-Harcourt
Department of Paediatrics
*Lecturer I
**Senior lecturer
Niger Delta University, Wilberforce Island, Bayelsa State
College of Health Sciences
Office of the Provost
***Professor
Correspondence: Dr. IO George.
E-mail: georgeolemoh@yahoo.com

Introduction

NEONATAL jaundice is a common paediatric problem in Nigeria,[1-3] accounting for nearly half of all neonatal admissions.[2] Neonates with severe jaundice often develop kernicterus which can result in death or irreversible brain damage in those that survive.[3,4] Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most important causes of neonatal jaundice in Far Eastern, Mediterranean and African countries including Nigeria.[5] Jaundice associated with G6PD deficiency is often provoked by exposure to iterogenic substances including naphthalene balls, menthol, aspirin, primaquine, herbal preparations, and fava beans.[6,7] G6PD, a cytoplasmic enzyme distributed in all cells,[8] catalyses the first step in the hexose monophosphate pathway, producing reduced nicotinamide adenine dinucleoride phosphate (NADPH). This co-enzyme is a hydrogen donor for reactions of various biochemical pathways including regeneration of the reduced form of glutathione which acts as scavenger for noxious oxidative metabolites in the cells.

G6PD deficiency has a vast and varying clinical spectrum ranging from mild anaemia to severe jaundice. The degree of haemolysis varies with the type of inciting agent, the quantity ingested or inhaled, and the severity of the enzyme deficiency in the patient. The severity of the disease can be modified by both genetic and environmental factors.[9] This can range from less severe disease as noted in Israeli Jews,[10] where hyperbilirubinaemia responds to phototherapy and exchange blood transfusion is rarely required, to severe morbidity and mortality as demonstrated in Nigerian neonates.[3,5,11]

The purpose of this study was to evaluate the severity of jaundice among G6PD deficient neonates and to determine factors influencing the severity of jaundice. These factors, if found, would be used to make recommendations and policies aimed at reducing morbidity and mortality.

Subjects and Methods

This was a prospective study of jaundice in babies aged within the first 28 days of life who were admitted to the Special Care Baby Unit (SCBU) of UPTH from June 1, 2006 to December 31, 2006. The Hospital, established in 1979, is the only tertiary hospital located in the metropolis of Port Harcourt, the capital of Rivers State. It serves as both general and referral centre for neighbouring states.

A general physical examination was carried out on all the babies admitted, with special reference to jaundice which was elicited by blanching the skin on the nasal bridge or tip of the nose with digital pressure exposing the underlying skin. A structured questionnaire was used to collect data on mother and neonate; such data included sociodemographics, exposure to iterogenic substances, history of peripartum pyrexia, prolonged rupture of foetal membranes, age at onset of jaundice, history of seizure, mode of treatment of jaundice and outcome. Two mls of venous blood withdrawn from each neonate was put in an EDTA bottle and immediately sent to the laboratory for quantitative enzyme assay. Laboratory investigations in each case included serum bilirubin level which was estimated using the Van den Bergh diazo reaction,[12] while G6PD assay was based on the method of Kornberg *et al*.[13] Microcrystalline cellulose was prepared by mixing 0.5gm of microcrystalline cellulose in 20mls of ice-cold saline. The red blood cells were isolated by filtration through microcrystalline mixture. The assays were carried out at a temperature of 30°C. Step 1: 100ml of 6mmol/l glucose-6-phosphate (G6P) was added to a test tube and mixed with 100ml of Tris-Hcl EDTA buffer with Ph 8.0; Step 2: 100ml of 100mmol/l $MgCl_2$ was added to the test tube and mixed thoroughly; Step 3: 20ml of 1:10 mmol/l haemolysate was added to the solution and mixed again; Step 4: 580ml of water was then added to the solution with thorough mixing. The steps were also taken for control test with the same reagents except for G6P where 680ml of water was added instead of 580ml. Both assay and control were read within five minutes of completion and the value of the control was eliminated by subtraction. The reading involved measurement of change in light absorbance at 340nm as the NADP was reduced to NADPH by G6PD. Solutions deficient in G6PD have little or no change in light absorbance at 340nm. The activity of the G6PD enzyme in the haemolysate was calculated from the initial rate of change of NADPH accumulation:

$$\text{G6PD activity in lysate (in mol/l)} = DA/\text{min} \frac{10^3}{6.22},$$

where 6.22 is the mmol extinction coefficient of NADPH at 340nm, 10^3 is the factor appropriate for the dilution in the reaction mixture and DA/min is the recorded peak ultraviolet light absorption at 340nm in the substrate divided by the time over which the absorption took place. Results were expressed as enzyme units per gram (eu/gmhb). The normal G6PD activity in adults is 8.83 ± 1.59 eu/gram haemoglobin at 30°C.[13]

Data were arranged in frequency tables and discrete variables were compared using Chi-square statistic, while continuous variables were compared using the Student t test. In all instances, a p value of less than 0.05 was regarded as statistically significant.

Results

Four hundred neonates were recruited for the study. They consisted of 288 (72.0 percent) males and 112 (28.0 percent) females. The male/female ratio was 2.6:1. Two hundred and eight (52.0 percent) were inborn while 192 (48.0 percent) were outborn. The mean ± (1SD) bilirubin level for the study population was 257.62 ± 83.μmol/1, while the means for neonates with G6PD deficiency and normal G6PD enzyme activity were 278.48 + 97.2 μmol/1 and 248.09 ±74.0 μmol/1, respectively (t = 3.5, P = 0.005).

In the quantitative analysis of G6PD enzyme activity, 210 (52.5 percent) neonates were deficient while 190 (49.5 percent) were G6PD normal. Of the G6PD deficient group, 145 (69.0 percent) were males while 65 (31.0 percent) were females giving a male/female ratio of 2.2:1. The mean ± (1SD) G6PD level of activity for all the deficient neonates was 17.3 ± 10.9 percent. Further analysis showed that the mean ± (1SD) for the deficient males and females were 12.4 +7.7 percent and 28.5 ± 8.5 percent respectively, a difference that was statistically significant (t = 19.9, P = 0.00). Table I shows the G6PD level of activity in male and female neonates. Eighty eight percent of males and 15.4 percent of females had G6PD activity level below the critical value of 20 percent; this difference was statistically significant (X^2 = 110.32; df = 3; p = 0.0000). Most of the 135 G6PD deficient neonates (64.3 percent) had severe jaundice (serum bilirubin level > 260μmol/l) as shown in table II.

Eighty four (40.0 percent) of the G6PD deficient neonates were exposed to iatrogenic substances; of these, 68 (50.4 percent) had severe jaundice while the jaundice was milder in 16 (21.3 percent). Of the 126 (60.0 percent) unexposed neonates, 67 (49.6 percent) had severe jaundice while 59 (78.7 percent) had milder jaundice. The difference in the severity of jaundice between neonates who were, and were not exposed was statistically significant (X^2 = 15.75; df = 1; p = 0.000723).

Table III shows the iatrogenic substances identified and their frequency in both G6PD deficient and non-deficient populations. While 54 (25.7 percent) of the G6PD deficient neonates were exposed to naphthalene balls, none were exposed to nalidixic acid or traditional herbs. Of the 140 male G6PD deficient subjects, 106 (78.5 percent) had severe jaundice while 39 (52.0 percent) had milder jaundice. Twenty nine (21.5 percent) of the 65 female deficient subjects had severe jaundice while 36 (48.0 percent) had milder jaundice. There was significant association between gender and severity of jaundice (X^2 = 14.65; df; p = 0.0013).

Table I

G6PD Activity Levels in Male and Female Neonates

G6PD Activity (%)	*Males n = 145(%)*	*Females n = 65(%)*	*Total n = 210 (%)*
< 10	61 (42.1)	3 (1.6)	64 (30.5)
10-20	67 (46.2)	7 (10.8)	74 (35.2)
21-30	12 (8.3)	22 (33.8)	34 (16.2)
31-40	5 (3.4)	33 (50.8)	38 (18.1)
Total	145 (100.0)	65 (100.0)	210 (100.0)

X^2 = 110.32 ; df = 3; p = 0.0000.

Figures in parentheses are percentages of the total

Table IV shows the outcome of treatment and G6PD status of the study population. One hundred and eighty-eight (89.5 percent) of 210 of the G6PD deficient subjects were discharged while seven (3.3 percent) developed kernicterus and 15 (7.2 percent) died.

Discussion

Neonatal jaundice is one of the most life-and health-threatening consequences of G6PD deficiency and kernicterus may result in these infants. In this study, the prevalence of G6PD deficiency among jaundiced

Table II

Bilirubin Levels and G6PD Status in the Study Population

Bilirubin Levels (µmol/l)	*G6PD deficient Neonates (%)*	*Normal G6PD Neonates (%)*	*Total (%)*
<175	42 (20.0)	30 (15.9)	72 (18.0)
175-260	33 (15.7)	85 (44.7)	118 (29.5)
>260	135 (64.3)	75 (39.4)	210 (52.5)
Total	210 (100.0)	190 (100.0)	400 (100)

$X^2 = 141.16$, df = 2, p = 0.000.

Figures in parentheses are percentages of the total

Table III

Iatrogenic Substances identified among the G6PD deficient and G6PD normal Groups

Iatrogenic Substances	*G6PD deficient*	*G6PD normal*	*Total*	*Percent*
Naphthalene balls	54	20	74	54.0
Oxytocin	22	23	45	32.9
Mentholated dusting powder	8	2	10	7.3
Native herbs	0	3	3	2.2
Nalidixic acid	0	5	5	3.6
Total	84	53	137	100.0

Table IV

Outcome of Treatment and G6PD status of the Study Population

Outcome	*G6PD deficient Neonates (%)*	*Normal (G6PD) Neonates (%)*	*Total (%)*
Discharged	188 (89.5)	170 (89.4)	358 (89.5)
Kernicterus*	7 (3.3)	10 (5.3)	17 (4.2)
Died	15 (7.2)	10 (5.3)	25 (6.3)
Total	210 (100.0)	190 (100.0)	400 (100.0)

Figure in parenthesis are percentages of the total

* Common features of kernicterus identified were poor suck, incomplete or absent moro reflex, abnormal tone, seizure and lethargy.

neonates was 52.2 percent. In keeping with the X-linked recessive inheritance[13] of G6PD deficiency, it was more prevalent in males.

The mean G6PD enzyme activity in the deficient subjects at 17.3± 10.9 percent is comparable with previously documented results in which mean values of 5-15 percent in the A- variant were obtained.[14] The variant G6PD A- is found predominantly in African Americans and is associated with moderate (class III) haemolysis in those who take primaquine. This variant (A-) has been documented to be responsible for all the deficiencies seen in Africa.[15] Other variants are G6PDA+ (found in 20-30 percent of black Africans but is not associated with haemolysis), the normal or wild type enzyme G6PD B (found in Caucasians, Asians, and the majority of African Americans) and the Mediterranean and closely related Asian variants (found in Caucasians from the Mediterranean basin and Asians, respectively) which are associated with severe (class II) haemolysis after primaquine administration.[15] The level of the enzyme activity in the present study was significantly lower in males than in females. More than 80 percent of the G6PD deficient males had enzyme levels of activity 20 percent and below the normal value which suggest severe enzyme deficiency and the likelihood of haemolysis on exposure to oxidative stress.[16] The severity of the enzyme deficiency may have accounted for the high frequency of severe jaundice among the G6PD deficient males in this study.

Forty percent of the G6PD deficient neonates in this study were exposed to iterogenic substances such as naphthalene balls, oxytocin drip and mentholated dusting powder. Naphthalene which was the major iterogenic substance encountered, is a bicyclic aromatic hydrocarbon with the chemical formula $C_{10}H_8$.[17] It is a natural constituent of coal tar and crude oil which is prevalent in Port-Harcourt, a centre for oil exploration.[17] It is used by consumers as deodorizers in toilets and diaper pails. Naphthalene can affect babies through contact by dermal absorption or through inhalation when it is absorbed rapidly into the blood stream. Individuals deficient in G6PD have been identified as being potentially sensitive to naphthalene exposure.[9] These individuals have low erythrocyte levels of reduced glutathione, a compound that normally protects red blood cells against oxidative damage. G6PD-deficient neonates, infants, and the foetus are particularly prone to naphthalene toxicity because the metabolic pathways responsible for conjugation of toxic metabolites (a prerequisite for excretion) are not yet well developed. In addition, they have low levels of methaemoglobin reductase, an enzyme that catalyzes the reduction of methaemoglobin, an oxidized form of haemoglobin that occurs in association with haemolytic anemia.[9]

Oxytocin use in labour is associated with the occurrence of neonatal jaundice.[9,12] In this study, 10.5 percent of G6PD deficient neonates were exposed to oxytocin during induction of labour. Various mechanisms have been advanced to explain this observation. They include trauma to foetal erythrocytes as a result of oxytocin induced hypertonicity,[18] vasoconstrictive action of oxytocin on uterine blood vessels, alterations in erythrocyte deformability due to the anti-diuretic activity of oxytocin,[19] and hyponatremia caused by the administration of large quantities of electrolyte-free fluids with oxytocin.[20] Also, 3.8 percent of the G6PD deficient neonates were exposed to mentholated dusting powder, a compound that contains menthol and camphor (naphthalene) known to cause haemolysis in G6PD deficient infants.[21] However, none of the G6PD deficient neonates were exposed to traditional herbs. This may be a reflection of the effects of health education given to the mothers

during antenatal care about the use and effects of traditional 'concoctions'.

The incidence of severe neonatal jaundice in G6PD deficient neonates varies with ethnic groups, while geographical differences exist among populations with the same ethnic background.[4] In this study, 64.3 percent of the G6PD deficient neonates had severe jaundice at the time of admission. It is possible that exposure of the babies to inhalational and dermal hydrocarbons from crude oil such as naphthalene, which abound in this area, might have been responsible for this high degree of severity of jaundice in the babies. This finding is similar to those reported by others[3,13] in which G6PD deficiency was shown to be associated with severe neonatal jaundice. However, this incidence is surprisingly high since the mutation G6PD A- that is responsible for nearly all the deficiency seen in Africans usually results in milder morbidities.[26]

In this study, 73.1 percent of the G6PD deficient males in contrast to 42.0 percent of the G6PD deficient females, had severe jaundice. This may be as a result of the fact that the males were homozygous and that the enzyme levels were lower in males. Furthermore, the enzyme disorder is X-linked which means that all the red blood cells of the deficient males are affected by the enzyme deficiency and would naturally be haemolysed when exposed to oxidative stress resulting in severe jaundice. It is not surprising therefore, that more males than females presented with severe jaundice, since only a smaller proportion of the red blood cells of the female are enzyme deficient which will result in milder haemolysis and jaundice except in the homozygous state.[2] This finding is consistent with those reported in other studies.[3,27]

Kernicterus has been described as a complication of G6PD deficiency-associated neonatal jaundice in many population groups.[3,5] In this study, poor suck, lethargy, incomplete or absent moro reflex, seizure and abnormal tone were found as clinical correlates of kernicterus which are in keeping with other reports.[1,11] The overall frequency of kernicterus was 3.3 percent among G6PD deficient neonates, a lower incidence than previously reported.[3,5] This could be explained on the basis of early presentation to the hospital and prompt treatment of cases.

In conclusion, we wish to stress that early detection of G6PD deficiency is important in reducing the risk of severe hyperbilirubinaemia, kernicterus and the need for exchange blood transfusion. This emphasizes the need for neonatal screening on cord blood samples for G6PD deficiency. Also, infants with G6PD deficiency should be protected from haemolytic agents such as naphthalene, oxidant drugs and menthol-ated powders. Parental education on neonatal hyperbilirubinaemia should highlight the causes and consequences of jaundice.

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